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3 ULTRASTRUCTURAL STUDY OF RAT LUNGS  
FOLLOWING INHALATION OF HIGH  
CONCENTRATIONS OF CS GRENADE SMOKE

by

H.F. Colgrave, and J.M. Creasey

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ULTRASTRUCTURAL STUDY OF RAT LUNGS FOLLOWING INHALATION  
OF HIGH CONCENTRATIONS OF CS GRENADE SMOKE

by

H.F. COLGRAVE and J.M. CREASEY

SUMMARY

The possible immediate and short term effects on lungs exposed to high concentrations of pyrotechnically generated CS smoke have been a matter of concern. An electron microscopical investigation has been undertaken to evaluate what lung changes, if any, follow early after exposure.

Three groups of 18 rats were exposed respectively to Cts of CS of 90,000, 60,000 and 30,000 mg min/m<sup>3</sup> after which animals were sacrificed at intervals from 15 minutes to 2 days. After death lungs were examined macroscopically, by electron microscopy and conventional histology.

Distress appeared to be maximal between 18-36 hours after exposure, when a total of 6 deaths occurred; 4 after a dosage of 90,000 mg min/m<sup>3</sup> and 2 after a dosage of 60,000 mg min/m<sup>3</sup>.

In animals that died there was severe congestion and areas of alveolar haemorrhage, with some interstitial oedema. Survivors showed less marked pulmonary congestion, haemorrhage and oedema.

Electron micrographs revealed changes to the alveolar epithelium and interstitium in the form of accumulation of fluid between the membrane layers and collagen containing areas of the septum. The presence of fluid was associated with degenerative changes of the epithelium and endothelium leading to rupture or dissolution of the capillary wall so allowing the extravasation of red blood cells and fluid into the alveolar space.

Rats that died after the exposure appeared to have acute pulmonary oedema; the lung damage in surviving rats as judged by appearances 2 days after exposure appeared to be transient.

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The cause of death of animals exposed to high concentrations of CS grenade smoke was acute pulmonary oedema associated with capillary damage.

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INTRODUCTION

$\alpha$ -Chlorobenzylidene malononitrile (CS) replaced  $\omega$ -chloroacetone-phenone (CN, CAP) as a riot control agent and as a training agent for a number of reasons including greater effectiveness and improved safety (Crichton, et al, 1958).

Punte et al (1962) determined the  $L(Ct)50$  of CS aerosol to various animal species and confirmed the high ratio between irritating and lethal concentrations. However, pure particulate CS is possibly less toxic when inhaled at high concentrations than is CS generated as grenade smoke. Such pyrotechnically generated smokes contain reaction products formed from the CS and pyrotechnic fuel for which detailed toxicological data are not available.

The use of CS in Vietnam (Kahn, 1968) caused concern regarding the possible effects on individuals exposed to high concentrations of CS grenade smoke, such as might be encountered in dug outs or other confined spaces. The use of the same agent more recently in Northern Ireland has reinforced this concern.

Inhalation of high concentrations of CS grenade smoke appears to cause direct injury to the pulmonary capillary endothelium, (Ballantyne, 1971; Ballantyne and Callaway, 1970, 1972; HMSO, 1971). This finding has been subjected to a detailed investigation especially into the damage to the pulmonary epithelium which may impair respiratory gas exchange.

The structure of normal lung is illustrated generally in Figures 1 and 2.

Electron micrographs of the normal alveolar wall show a continuous lining membrane. Separating adjacent alveolar membranes, but intimately associated with them, are capillary spaces separated only by the basement membrane of the epithelium and endothelium of the capillary from the alveolar space. Therefore, respiratory gases traverse a barrier

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comprising (1) alveolar epithelium, (2) basement membrane, (3) endothelium of the pulmonary capillary (Fig 3). The thickness of the pulmonary epithelium averages about 0.2-0.7  $\mu\text{m}$ . In the region of the nucleus of the epithelial (type I) cell, it is about 4  $\mu\text{m}$  thick, and the cytoplasm attenuates to form an uninterrupted layer along the surface of the alveolar wall to the next capillary (Fig 4). In small animals, e.g. mouse, the epithelium may be as thin as 0.025  $\mu\text{m}$  (Karrer, 1956).

There are two types of cells at the alveolar surface. The alveolar (type II) cell is a large cell situated in the alveolar cleft adjacent to the capillary in the thickened areas of the wall. This cell often has numerous lamellar osmiophilic inclusion bodies which are believed to be the source of the lung surfactant (Pattle, et al (1972a)) and it shows microvilli facing the alevolar space. These bodies are usually excavated or vacuolated when ethanol is employed, during processing, as a dehydrating fluid. Type II epithelial cells are also capable of proliferation by metamorphosis to form at least one type of alveolar macrophage, which becomes free and more abundant during infections, and after exposure to noxious fumes and irritant particles.

The normal alveolar lining is at its thinnest around the capillaries where gaseous exchange is taking place; the capillary lumen normally contains a few red cells. The known reactions following exposure to chemical irritants of the triple structured membrane described above are thickening, oedema and sometimes ballooning away from the basement membrane (Kisch, 1958; Bils, 1966, 1967, 1970; Cottrell, 1967), thus impairing gaseous exchange. In view of this the capillary membranes, in the present investigation, were especially scrutinized for damage in addition to a more general search for abnormalities.

MATERIAL AND METHODS

Three groups of male albino S.P.F. rats, each of 18 animals were exposed to pyrotechnically-generated CS smoke in a 10  $\text{m}^3$  chamber from 4 CS cartridges (L3A1), each containing 12.5 g of CS, 15 g potassium chlorate, 15 g of lactose and 7.5 g kaolin.

Group 1 was exposed for 15 minutes to a mean concentration of 6  $\text{g/m}^3$ . The average concentration being calculated by sampling the cloud every minute during exposure. The dosage to which this group was exposed was 90,000 mg  $\text{min}/\text{m}^3$  (Table 1), expressed as the product of concentration and time (Ct).

Group 2 was exposed under similar conditions to a mean concentration of 6  $\text{g/m}^3$  for 10 minutes i.e. a Ct of 60,000 mg  $\text{min}/\text{m}^3$  (Table 2).

Group 3 was similarly exposed for 5 minutes to a mean concentration

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of  $6.4 \text{ g/m}^3$ , i.e. a dosage of  $32,000 \text{ mg min/m}^3$ . (Table 3).

After the exposure animals were immediately removed into fresh air. Any animals dying were immediately examined and tissue taken for conventional histology and electron microscopy. Survivors were sacrificed by an intraperitoneal injection of 6% sodium pentobarbitone (Nembutal), at times ranging from 15 minutes to 2 days. Details of times of killing are shown in Table 4.

Postmortem and Histology

Lungs, livers and kidneys of all animals were examined macroscopically for evidence of such changes as congestion, emphysema and haemorrhage. Pieces of all three organs, intended for conventional histology, were fixed in 10% formalin in neutral phosphate buffer, then embedded in paraffin wax. Sections were stained with the following methods; haematoxylin and eosin, periodic acid Schiffs (Pearse, 1960), and the technique of Schmorl for incipient fibrosis, (Carlton, and Drury, 1957).

Electron microscopy

Lung tissue was taken from affected areas and slices of 3 mm thickness were cut and fixed in 2.5% buffered glutaraldehyde at pH 7.4 for 2 hours at  $+4^\circ\text{C}$  (Karlsson and Schultz, 1965). Following overnight immersion in 0.1 M cacodylate buffer at pH 7.4, tissue was chopped into 1 mm cubes and post fixed in 1% osmium tetroxide at pH 7.4 for 2 hours at  $+4^\circ\text{C}$  (Millonig, 1961). The final five steps in preparation involved rinsing in cacodylate buffer and then in distilled water and dehydrating in alcohol (ethanol); propylene oxide was then used as a clearing agent before embedding in Araldite (Luft, 1961). Blocks were cut on a Cambridge Huxley Ultramicrotome using glass knives made on the L.K.B. Knifemaker and supported in the microtome by the modified knife holder of Marshall and Sheen (1970). Sections were mounted on copper grids, without supporting film, stained with 5% uranyl acetate for 1 hour at  $60^\circ\text{C}$  (Pease, 1964), then lead citrate (Reynolds, 1963) for a few minutes, rinsed with 0.02 N NaOH followed by a rinse in distilled water and blotted dry. Examination and micrography were undertaken with a Philips EM 300 Electron Microscope at either 60 Kv or 80 Kv according to the available contrast in the sections.

RESULTS

Immediately after removal to the fresh air from the  $10 \text{ m}^3$  chamber all animals lacrymated and had blood stained noses. Four rats died 24 hours after a dosage of  $90,000 \text{ mg min/m}^3$  and 2 rats died after a

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dosage of 60,000 mg min/m<sup>3</sup>, one at 24 hours and one at 36 hours. There were no deaths amongst the rats having a dosage of 30,000 mg min/m<sup>3</sup>, (Table 4).

Autopsy and histological findings

Microscopically the lungs of nearly all rats, irrespective of whether they died or were sacrificed within 48 hours, showed variable degrees of congestion, haemorrhage and oedema, with froth exuding from the cut surfaces. The lungs of rats that died were slightly more severely affected than those sacrificed (Figs 5 and 6).

Details of histological lesions in individual rats that died or were sacrificed from all three groups are given on Tables 5, 6 and 7.

1. Group 1 animals (Ct = 90,000). The animals that died had marked congestion of alveolar capillaries and intrapulmonary veins, with scattered alveolar haemorrhage and patchy oedema. Animals that were sacrificed had much less marked congestion of the lung, and only occasional areas of haemorrhage and oedema. Apart from occasional mild congestion of the kidneys and livers these organs were entirely normal.
2. Group 2 animals (Ct = 60,000). The 2 rats that died had congestion of the alveolar capillaries, but this was considerably less marked than in those dying in Group 1. Haemorrhage and oedema were only occasionally found in rats of Group 2. Survivors sacrificed up to 2 days showed mild congestion of the alveolar capillaries, and very occasional alveolar haemorrhages. Apart from minimal congestion of the livers and kidneys in those surviving and dying, these organs were completely normal.
3. Group 3 animals (Ct = 32,000). In general, up to 6 hours after exposure the lungs demonstrated mild congestion with occasional alveolar haemorrhage, but no oedema. With the exception of one animal, sacrificed at 2 days after exposure, the lungs of those sacrificed at 12 hours and onwards were entirely normal; kidneys and livers showed no abnormalities.

Electron microscope findings

During the initial screening of the lung sections under the electron microscope many membranes appeared diffuse or obscure. With the aid of the goniometer stage, such appearances could be reliably evaluated (Figures 7a and 7b).

This study of the fine structure of the lung after exposure to CS grenade smoke revealed some morphological alteration to the interstitium and of the epithelium and endothelium. Changes found in the blood-air barrier and interstitial layers of the septum of animals that died or were sacrificed were similar in all three groups, varying only in the degree

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of severity. In the high concentrations there was evidence of damage as early as 15 minutes after exposure, by 30 and 60 minutes damage was a little more severe.

Congestion of the alveolar vessels and intrapulmonary veins was a common finding in the histological sections and the electron micrographs showed localised detachment of the epithelium and endothelium, with evidence of rupture of the capillary wall (Figures 8 to 11). The formation of 'ballooning', only found occasionally in normal animals, occurred approximately 12 hours after exposure and was seen in the lungs of all exposed animals. This endothelial 'ballooning' usually occurred in the thinnest area of the capillary wall and was seen as a loop, sometimes filling a good deal of the lumen, or detached, forming a complete circle of endothelium free in the capillary (Figures 12, 13 and 14).

Intra-alveolar haemorrhages were seldom seen in any of the micrographs examined, but early evidence of capillary rupture or dissolution of the capillary wall was frequently seen (Figures 8, 15, 16 and 17).

Fluid in the collagen containing areas of the septum was responsible for separating the tightly packed fibres and thickening of the endothelial layer. However, the junctions between the attenuated cytoplasm of adjacent endothelial cells seemed intact and the bands of elastic tissue remained unaltered.

In the badly affected animals the epithelium and endothelium were swollen and separated from their basement membrane by fluid, (Figures 18 and 19), leaving 'cytoplasmic fragments' each with a limiting membrane in areas of separation (Figures 20 and 21). Increased pinocytosis was associated with the fluid between the epithelium and endothelium of the capillary wall (Figures 22, 23 and 24), resulting in the dissolution of the epithelium expelling microvesicles into the alveolar space, (Figure 15). In cases of damage to the capillary wall seen as detachment or dissolution of membranes by fluid, the basement membrane usually remained intact. In the lungs of the animals that died there was a complete disruption of the pulmonary epithelium, the fine capillaries were packed with red blood cells, and numerous white cells and active macrophages were present in the alveoli, (Figures 25 and 26). The lungs of those animals that survived 2 days after exposure showed little evidence of damage either by rupture of the capillary walls or by fluid in the interstitium or between the epithelium and endothelium. However, occasional 'ballooning' of the endothelium was seen. The greatest damage was found in the lungs of rats exposed to the highest dosages of CS grenade smoke and occurred soon after the completion of exposure but there was little to choose

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between the concentrations of group 1 and group 2, with deaths after exposure at 18-36 hours when animals from all three groups reached a period of maximum distress. After this period there were no deaths and the animals slowly recovered. Animals sacrificed at 2 days showed minimal changes on both conventional and electron microscopy and these findings are taken to imply the complete reversibility of such changes as have been observed in the acute stages of intoxication.

DISCUSSION

The animals in this study were exposed to a concentration of 6 g/m<sup>3</sup> in pyrotechnically generated CS smoke for 15, 10 or 5 minutes so that animals received dosages of 90,000, 60,000 and 30,000 mg min/m<sup>3</sup> CS respectively. These figures represent very much higher doses than would be encountered in a riot for it has been found that individuals exposed to CS smoke in the open will flee to fresh air after one minute's exposure to a concentration of say 10 mg/m<sup>3</sup>. Only when exposed persons are unable to escape and are exposed to improbably high concentrations could the conditions of this investigation begin to be approached. Nevertheless, the information obtained by this investigation is of considerable value in completing an extensive biological assessment of the safety of CS aerosols.

The histological appearances of the lungs observed in this study were similar to those found by Ballantyne, (1971), and Ballantyne and Callaway, (1970 and 1972) in their investigations with CS grenade smoke.

The electron microscope findings described above are in general agreement with similar results reported by other workers in acute pulmonary oedema in animals. Studies by Cottrell et al (1967) compared the effects of haemodynamic and Alloxan induced pulmonary oedema. They found that in the former the accumulation of fluid was interstitial in location and focal in nature, whereas in changes found in the Alloxan induced pulmonary oedema, damage was diffuse and not limited to the collagen containing areas of the interstitial space but was associated with degenerative changes to the epithelium and endothelium, a common finding with various other toxic and irritant agents.

Emphysema and impaired pulmonary function in laboratory animals was reported by Bils, (1966, 1967 and 1970) after exposure to high levels of gaseous constituents of smog notably, nitrous oxide, sulphur dioxide, phosgene, ozone, hydrocarbons and automobile exhaust. He found evidence of damage in the lungs of these animals in the form of 'ballooning' of the endothelium, swelling of the epithelium and endothelium and signs of oedema. However Bils (1966) also described an increase in activity of the alveolar

cell Type II in producing the lipoproteinaceous surface active material but there was no evidence of this in the micrographs after high dosages of CS smoke. An investigation is currently taking place by Pattle (1972b) and a fuller account will be published later.

In experimentally produced pulmonary oedema using intravenous and intramuscular suprarenin, Kisch (1958) reported changes to the epithelium and endothelium with rupture and dissolution of the capillary wall permitting the escape of liquid and blood cells. This work on rabbits and that of Messon and Schulz (1957), using an intraperitoneal injection of thiosemicarbocyanine to produce pulmonary oedema in rats, indicated that an abundant secretion of droplets by the epithelial cells of the smallest air ducts contribute to the liquid production in the lungs during acute pulmonary oedema.

This study confirmed the histological evidence that interstitial oedema was present. However, electron micrographs showed that fluid was first apparent in the collagen containing areas of the septum before entering the interstitium and permeating between epithelium and endothelium; little or no fluid was seen in the alveolar spaces.

Cottrell et al (1967) proposed that the collagen containing areas of the alveolar septum could serve as a reservoir to collect excess fluid which enters the interstitial space. It may be presumed that these connective tissue areas not only provide the structural framework of the lung but act as a sponge, keeping the alveoli in a dry state. In conditions of acute pulmonary oedema when the tissue is saturated, perfusion of fluid permits seepage between the epithelium and the endothelium of the capillary wall.

Unidentified membrane bound (cytoplasmic fragments) forms and pinocytotic vesicles were present in areas of separation of the epithelium and endothelium from their respective basement membranes.

Low (1953) in some electron microscopical studies of rat lung demonstrated the presence of thick and thin regions of the capillary wall, the thinnest regions being adjacent to the alveolar space. In a study carried out on the rat lung by Weibel and Knight, (1964), using a mathematical model representing a rippled or corrugated membrane, they demonstrated that the thicker regions of the blood air barrier accounts for only 15% of the overall gas conductance. In this investigation it was noted that the 'ballooning' of the capillary endothelium, also observed by Kisch (1958) in animals found in a moribund state with pulmonary oedema as well as in other conditions such as the final stages of scurvy in guinea pigs, was at its thinnest area. Although some of the capillaries appeared

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normal, even in animals that were dying, the infusion of fluid, detachment of the epithelium and 'ballooning' of the endothelium of the capillaries, especially in their thinnest and therefore normally most efficient region, could impede gaseous exchange giving rise to hypoxia and death.

It is the authors' view that extremely high dosages of CS grenade smoke cause pulmonary oedema with associated changes to both epithelium and endothelium such as separation of cells, 'ballooning' of the endothelium and dissolution of membranes giving rise to capillary damage.

CONCLUSION

This study showed that animals dying after exposure to very high concentrations of CS grenade smoke had congestion and severe capillary damage giving rise to pulmonary oedema and haemorrhage probably resulting in hypoxia and death; lung appearances of animals which survived the period of maximum distress suggests that the induced damage is transient.

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TABLE 1

GROUP 1

Concentration of CS in samples of cloud taken from the  $10\text{m}^3$  chamber during exposure.

Time of sampling (mins)	2	3	4	5	6	7	8	9	10	11	12	14
Concentration of CS mg $\text{m}^{-3}$	5,700	6,500	6,300	5,550	6,400	6,400	6,000	5,850	5,350	4,850	6,000	5,550

Mean concentration of CS over 15 minutes exposure =  $6,000 \text{ mg/m}^3$

$$C_t = 6,000 \times 15 = 90,000 \text{ mg min/m}^3$$

TABLE 2

GROUP II

Concentration of CS in samples of cloud taken from the  $10\text{m}^3$  chamber during exposure.

Time of sampling (mins)	1	2	3	4	5	6	7	8	9	10
Concentration of CS mg $\text{m}^{-3}$	6,150	6,350	5,200	5,650	7,400	5,550	5,500	5,850	6,200	6,400

Mean concentration of CS over 10 minutes exposure =  $6,000 \text{ mg/m}^3$

$$C_t = 6,000 \times 10 = 60,000 \text{ mg min/m}^3$$

TABLE 3

GROUP III

Concentration of CS in samples of cloud taken from the  $10\text{m}^3$  chamber during exposure.

Time of sampling every 30 seconds (mins)	1	$1\frac{1}{2}$	2	$2\frac{1}{2}$	3	$3\frac{1}{2}$	4	$4\frac{1}{2}$	5
Concentration of CS mg $\text{m}^{-3}$	7,300	5,100	7,300	7,300	6,900	6,500	7,100	7,800	8,900

Mean concentration of CS over 5 minutes exposure =  $6,400 \text{ mg/m}^3$

$$C_t = 6,400 \times 5 = 32,000 \text{ mg min/m}^3$$

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TABLE 4

Number of animals in group 1, 2 and 3 exposed to high concentrations of CS grenade smoke, showing times of sacrifice and times to death. Every group had 3 non exposed animals

		Number of animals and times to death											
Ct mg min/m <sup>3</sup>	Total No of animals exposed	Mode of death	15 mins	30 mins	1 hours	6 hours	12 hours	24 hours	30 hours	36 hours	2 days	Mortality Rate	% Mortality
90,000	18	Sacrificed	3	3	3	1	0	0	0	0	1	4/18	22%
		Died					4						
60,000	18	Sacrificed	3	3	3	1	1	1	0	1	1	2/18	11%
		Died						1					
32,000	18	Sacrificed	3	3	3	1	2	0	0	3		0/18	0%
		Died											

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TABLE 5  
Details of pathology of tissue taken from rats exposed to CS grenade smoke Ct 90,000 mg min/m<sup>3</sup> - GROUP I

Time of death / sacrificed	LUNG			KIDNEY			LIVER	
	Congestion	Haemorrhage	Oedema	Cortical necrosis	Medullary necrosis	Congestion	Congestion	necrosis
Sacrificed 15 mins	±	-	-	-	-	-	-	-
Sacrificed 30 mins	±	-	-	-	-	-	-	-
Sacrificed 1 h	+	-	-	-	-	-	-	-
Sacrificed 6 h	+	-	-	±	-	-	-	-
Sacrificed 12 h	-	+	-	±	±	-	-	±
Died 18 h	++	-	-	-	-	±	-	-
Died 24 h	+++	+	±	-	-	-	-	-
Died 24 h	+++	+	±	-	±	-	-	-
Sacrificed 2 days	+	-	-	±	-	-	-	-
Controls	-	-	-	-	-	-	-	-

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KEY - = negative  
 ± = mild and scattered  
 + = mild but extensive  
 ++ = moderate and extensive  
 +++ = severe

TABLE 6

Details of pathology of tissue taken from rats exposed to CS grenade smoke Ct 60,000 mg min/m<sup>3</sup> - GROUP II

Time of death/ sacrificed	LUNG			KIDNEY			LIVER	
	Congestion	Haemorrhage	Oedema	Cortical necrosis	Medullary necrosis	Congestion	Congestion	necrosis
Sacrificed 15 min	±	-	-	-	-	-	-	-
Sacrificed 30 min	-	-	-	-	-	-	-	-
Sacrificed 1 h	+	-	-	-	-	-	-	-
Sacrificed 6 h	-	-	-	-	-	-	-	-
Sacrificed 12 h	+	-	-	-	-	-	-	-
Died 24 h	++	+	+	-	-	-	-	-
Sacrificed 24 h	+	-	-	-	-	-	-	-
Sacrificed 30 h	+	-	-	-	-	-	-	-
Died 36 h	+	-	-	-	-	-	-	-
Sacrificed 2 days	+	-	-	-	-	-	-	-
Controls	±	-	-	-	-	-	-	-

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TABLE 7

Details of pathology of tissue taken from rats exposed to CS grenade smoke Ct 32,000 mg min/m<sup>3</sup> - GROUP III

Time of death/ sacrificed	LUNG			KIDNEY			LIVER	
	Congestion	Haemorrhage	Oedema	Cortical necrosis	Medullary necrosis	Congestion	Congestion necrosis	
Sacrificed 15 min	±	-	-	-	-	-	-	-
Sacrificed 30 min	±	-	+	-	-	-	-	-
Sacrificed 1 h	±	-	-	-	-	-	-	-
Sacrificed 6 h	±	-	±	-	-	-	Papilla	-
Sacrificed 12 h	-	-	-	-	-	-	-	-
Sacrificed 24 h	-	-	-	-	-	-	-	-
Sacrificed 2 days	-	++	-	-	-	-	-	-
Controls	-	-	-	-	-	-	-	-

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Figure 1. Diagram showing the general structure of the lung

1. Trachea
2. Main Bronchus
3. Terminal Bronchiole
4. Respiratory Bronchiole
5. Alveolus

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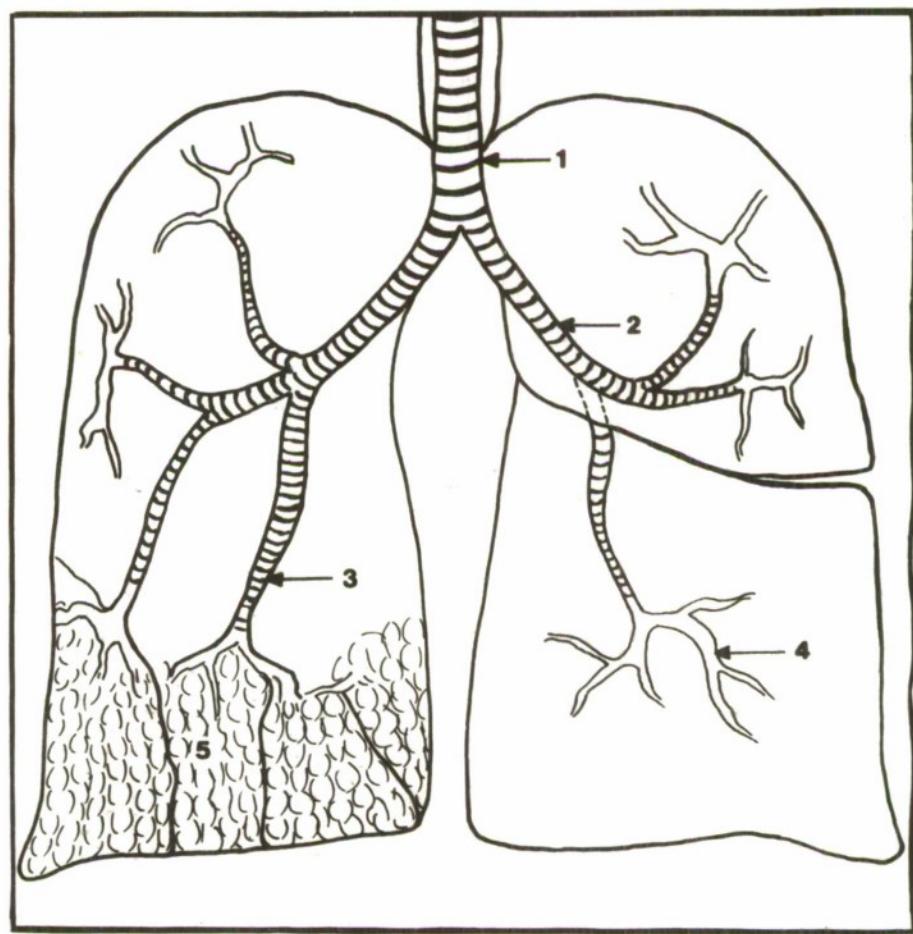


FIG. 1.

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Figure 2a. Diagram of the alveolus depicting the detailed architecture and the relationship of the capillaries to the alveolar air space.

Alv. S.	Alveolar space
Alv.C.Type I	Alveolar cell type I
Alv.C.Type II	Alveolar cell type II
B.M.	Basement membrane
Cap.	Capillary
C.	Collagen
E.	Elastic fibres
End.	Endothelium
Epth.	Epithelium
M.	Macrophage
Rbc	Red blood cell
End.C	Endothelial cell

Figure 2b. Electronmicrograph of a normal alveolus.

Magnification X 4,400

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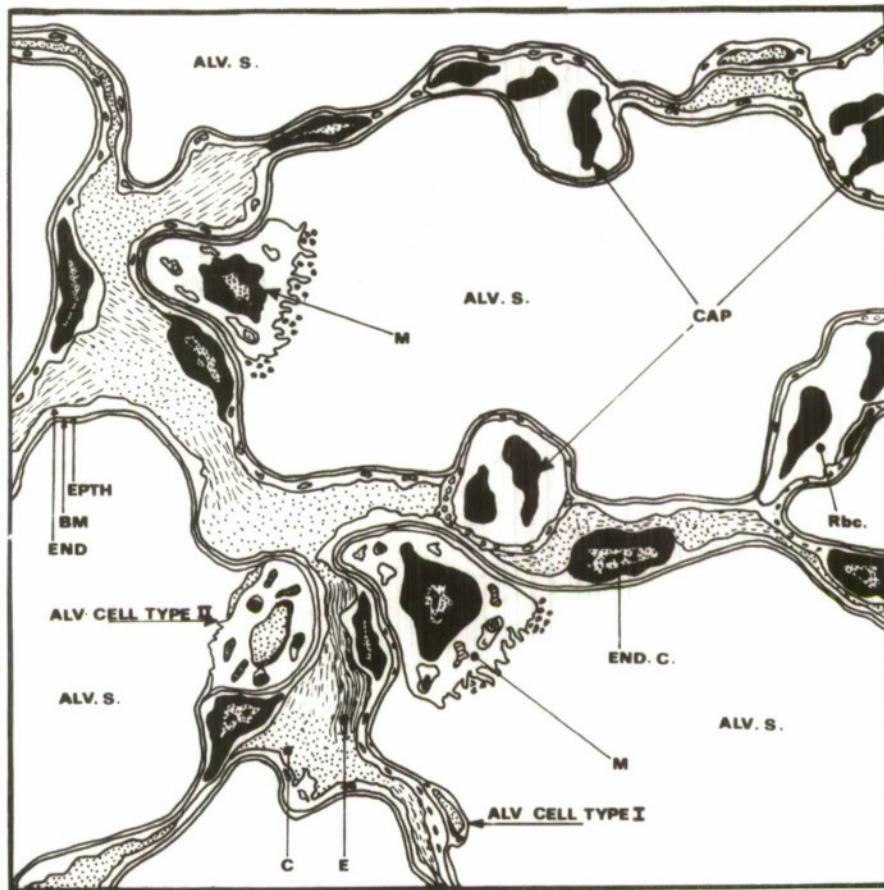


FIG. 2a.

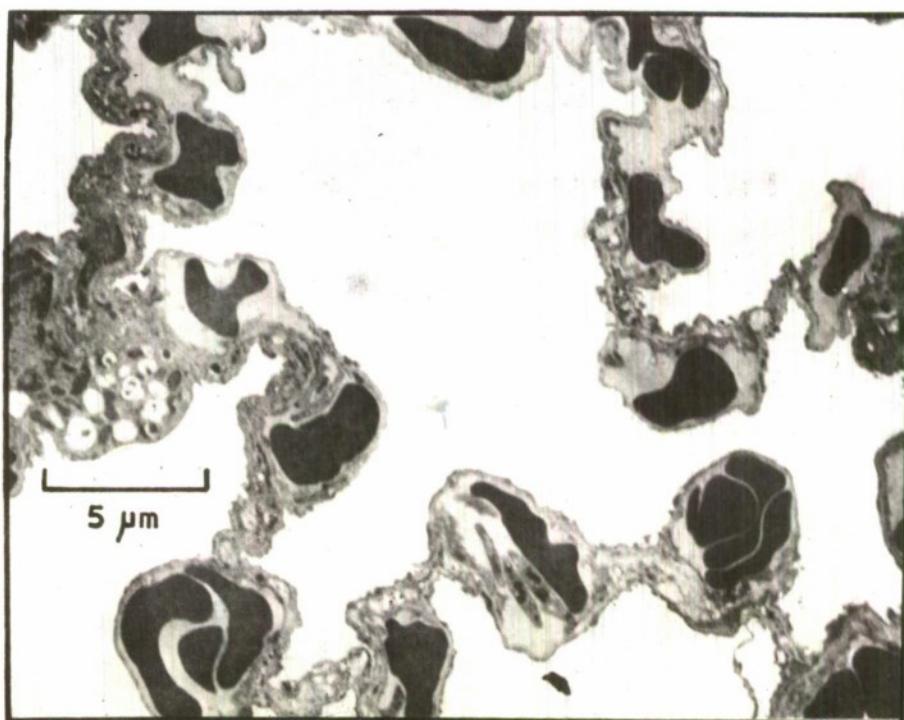


FIG. 2b.

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Figure 3. Normal alveolar epithelium showing the triple structured membrane.

Magnification X 45,000

A.E. Alveolar Epithelium  
C.E. Capillary Endothelium  
B.M. Basement Membrane  
P.V. Pinocytotic vesicles  
Wbc. White blood cell

Figure 4. Normal rat lung

Magnification X 2,700

Rbc. Red blood cell  
C. Capillary  
M. Macrophage

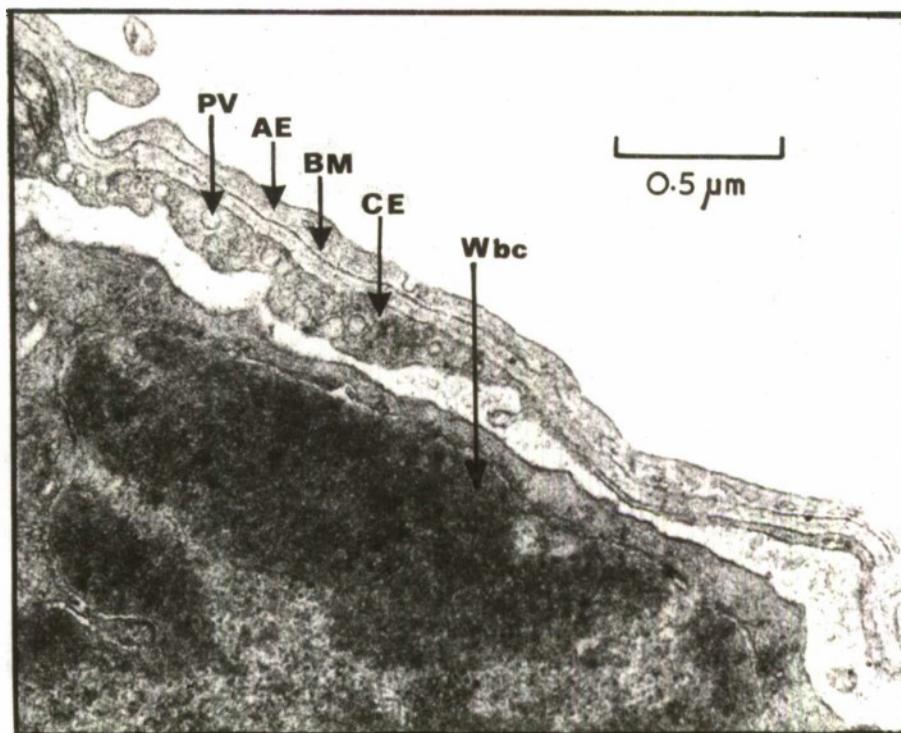


FIG.3.

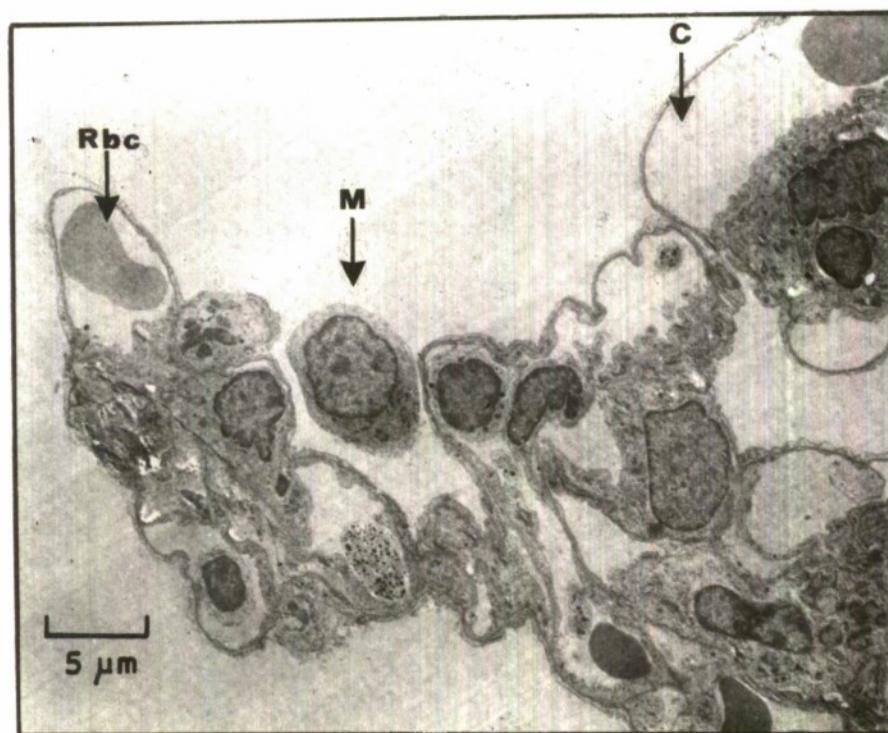


FIG.4.

Figure 5. Histological section of normal rat lung stained by  
H.E. 5 $\mu$  thick.  
Magnification X 340

Figure 6. A section of lung showing congestion and haemorrhage from  
an animal killed 12 hours after exposure to a Ct 90,000  
mg min m<sup>-3</sup>.  
Magnification X 340.

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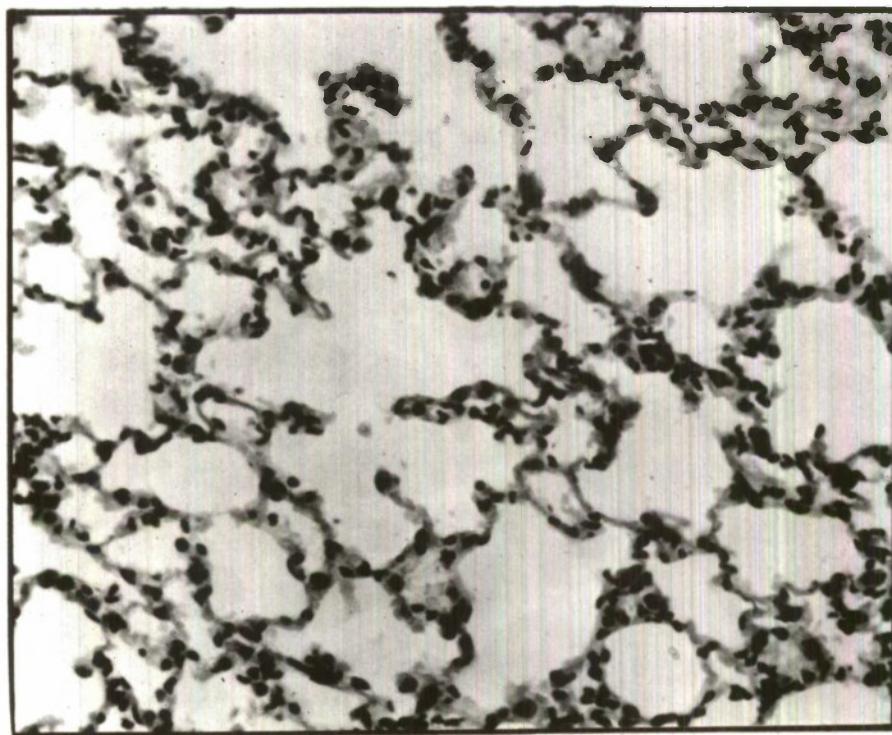


FIG.5.

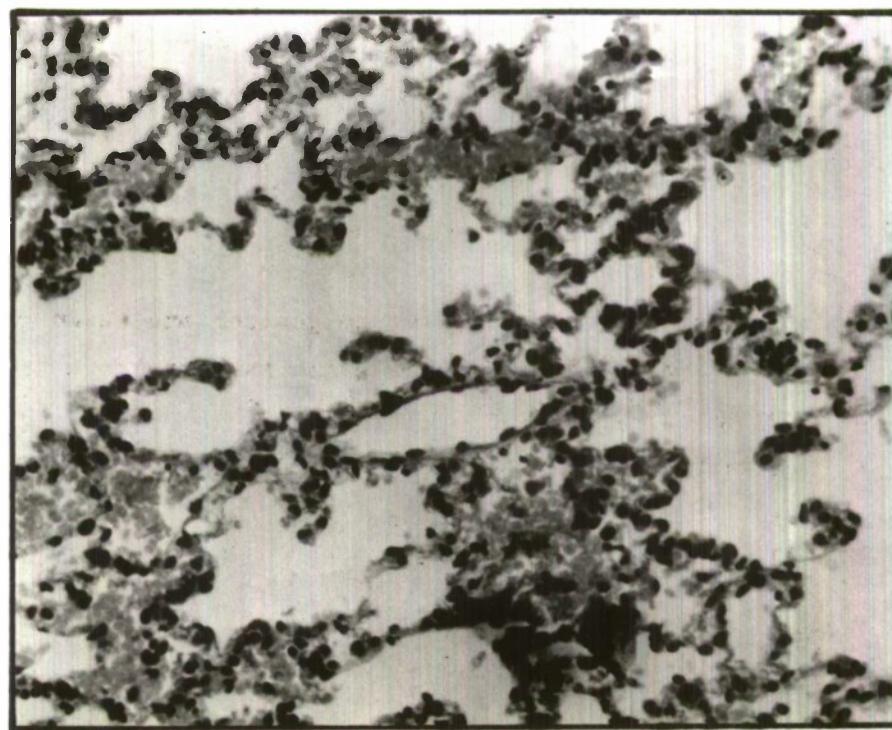


FIG.6.

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Figure 7a. Capillary membranes. No tilt

Magnification X 22,000

D.M.

Diffuse membrane

Appearance of capillary membranes before and after tilting the specimen.

Figure 7b. Capillary membranes Tilt 51°

Magnification X 22,000

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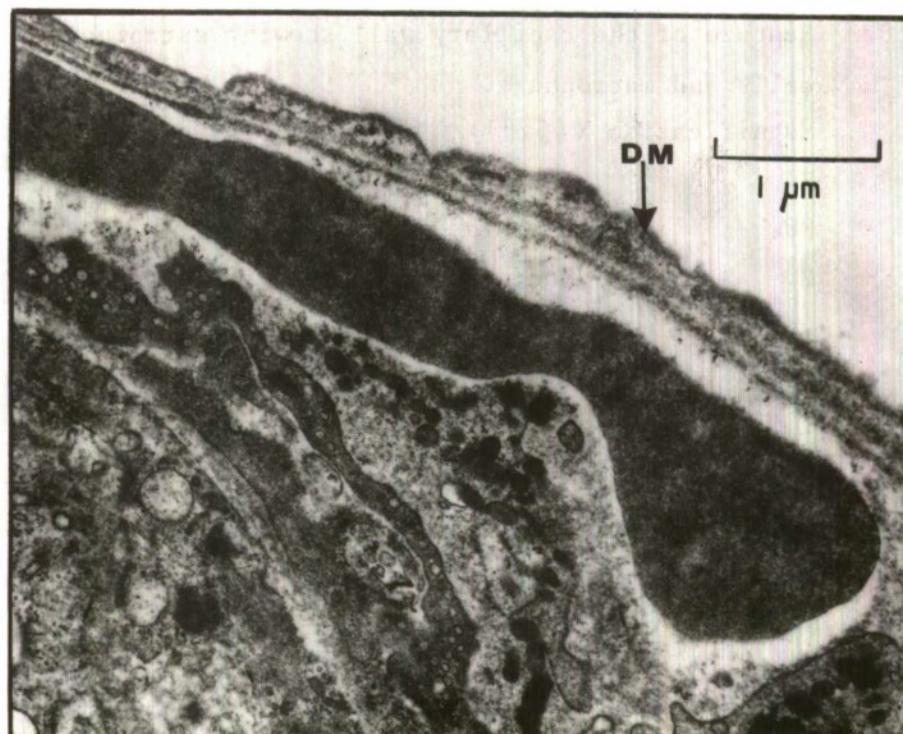


FIG. 7a.

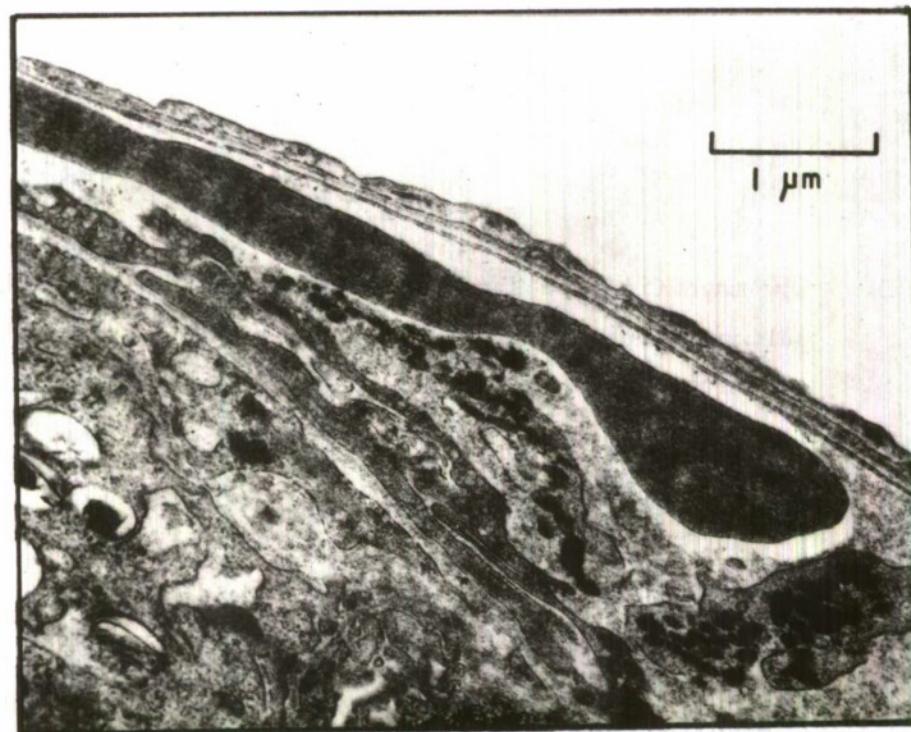


FIG. 7b.

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Figure 8. Rupture of the capillary wall showing escaping red blood cells and macrophage.

Magnification X 6,800

E Erythrocyte  
RW. Ruptured wall  
M. Macrophage

Figure 9. Low magnification showing areas of detachment of the pulmonary epithelium and free red blood cells in the alveolar space.

Magnification X 1,400

D. Areas of detachment  
E. Free erythrocytes in the alveoli  
M. Macrophages

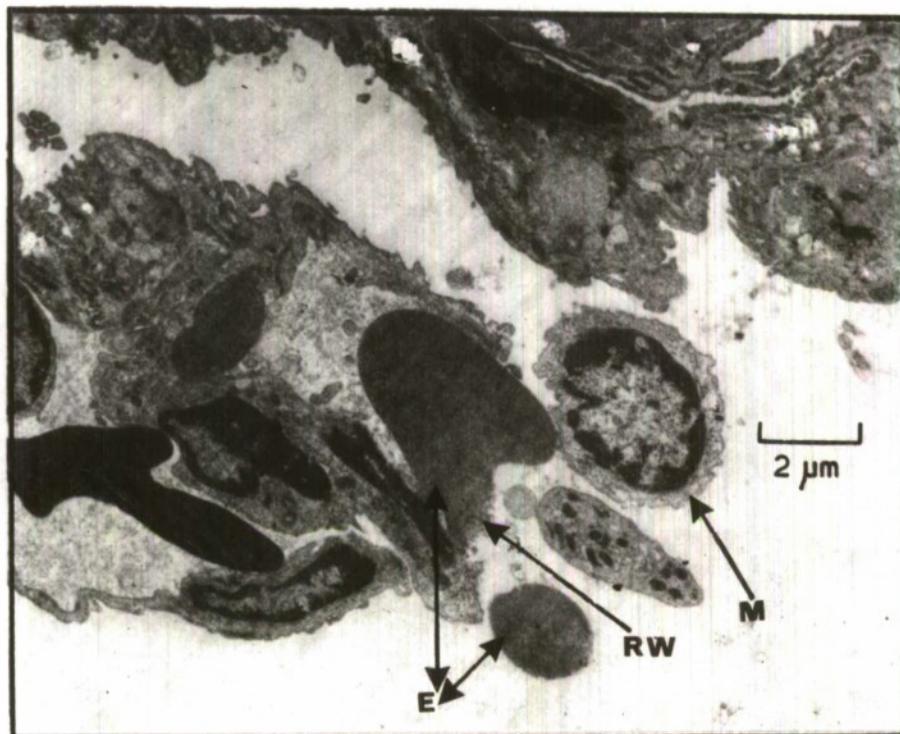


FIG. 8.

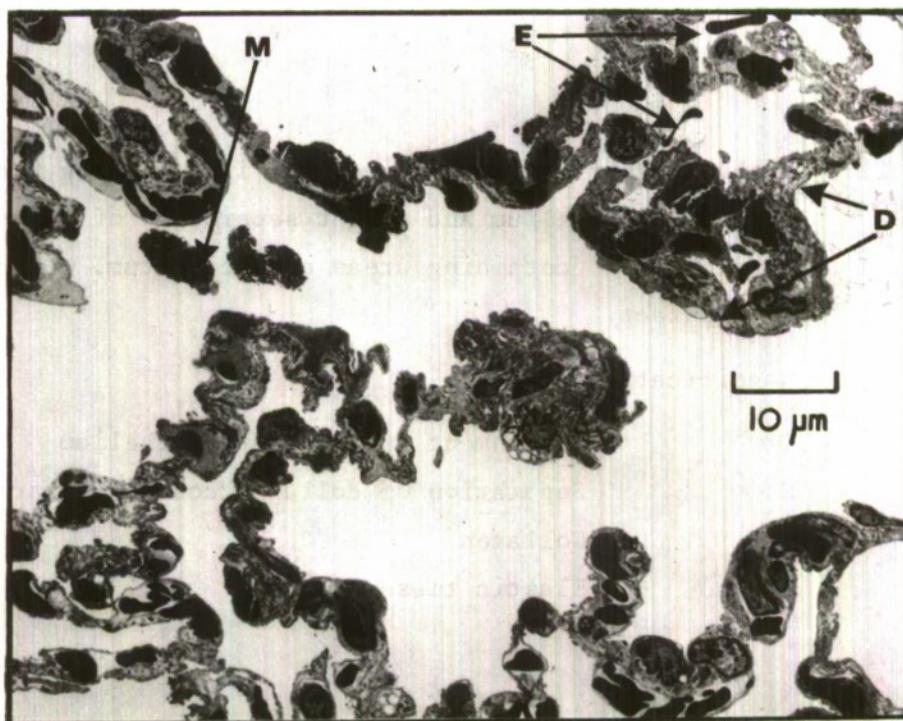


FIG. 9.

Figure 10. Area of localised epithelial detachment

D. Area of detachment

Magnification X 6,800

Figure 11. Higher magnification of figure 10 illustrating the detached epithelium and slight separation of the endothelium and collagen containing areas of the septum. Elastic fibres seem intact.

Magnification X 33,000

D Detachment of alveolar epithelium

S Separation of collagen containing areas of the septum

C Collagen

E Elastic tissue

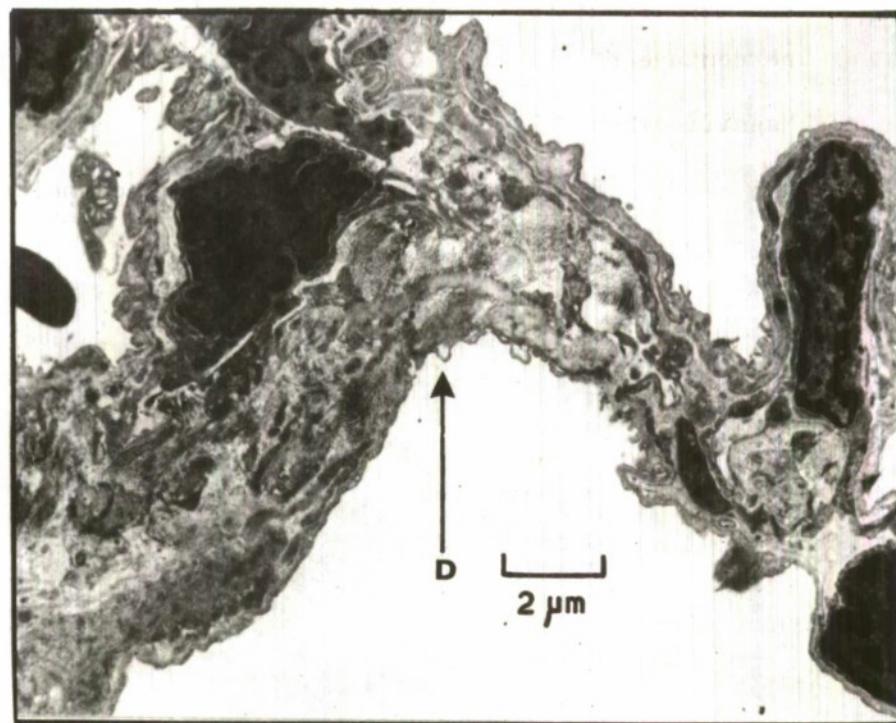


FIG. 10.

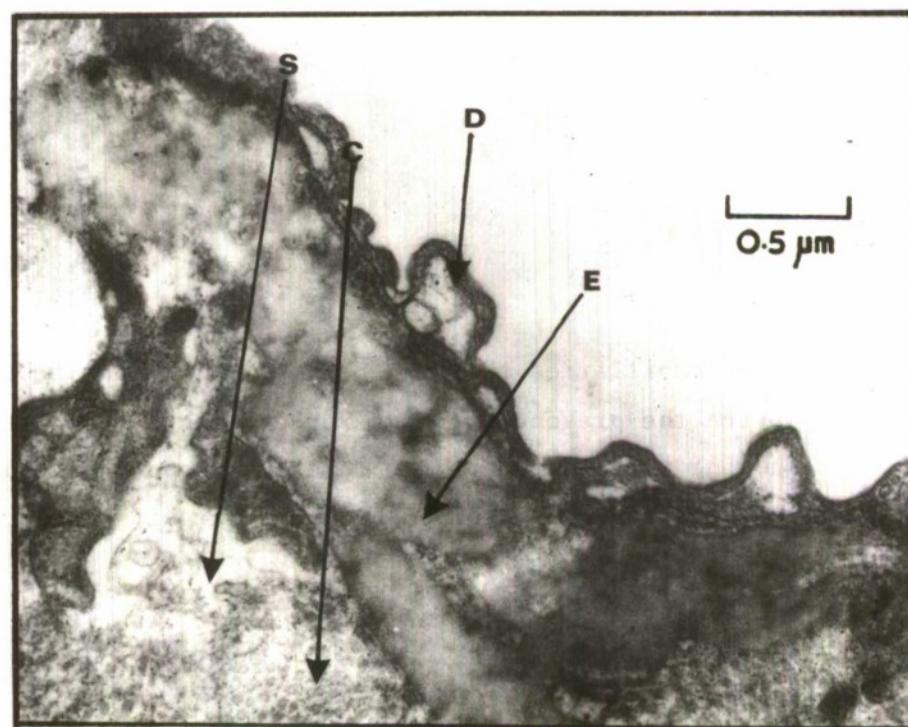


FIG. 11.

Figure 12. Low magnification showing 'ballooning' of a high percentage of the capillaries in the area shown.

Magnification X 1,400

B                  Ballooning of the capillary endothelium

Figure 13. Three capillaries showing 'ballooning' of the endothelium.

Notice the presence of a macrophage and a Type II cell showing vacuolation of the lamellated osmophilic bodies which have been dissolved by the processing methods.

Magnification X 4,800

B                  Ballooning of the capillary endothelium

M                  Macrophage

Alv.c              Alveolar cell Type II

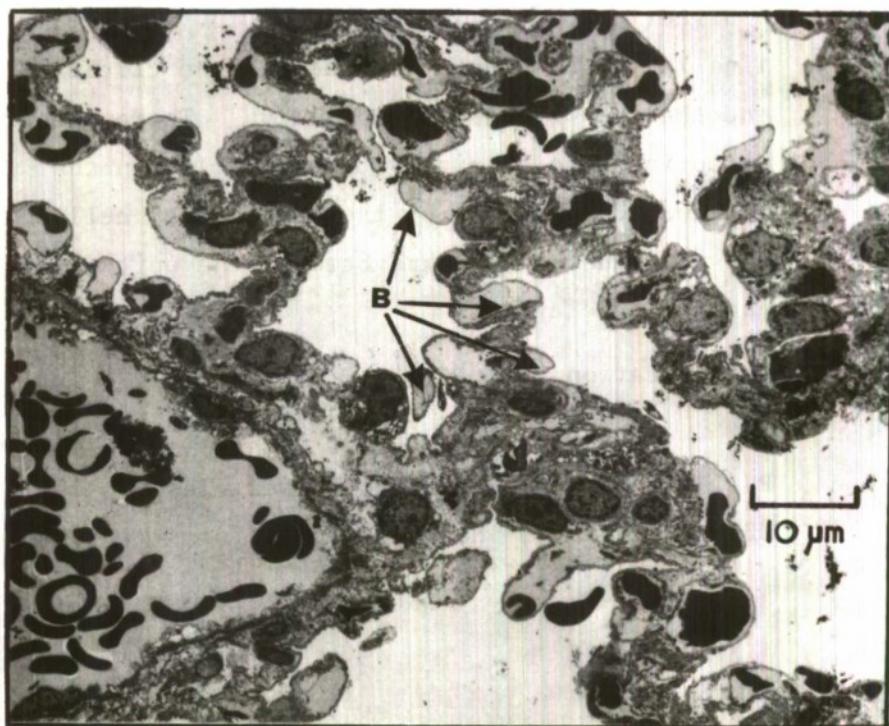


FIG. 12.

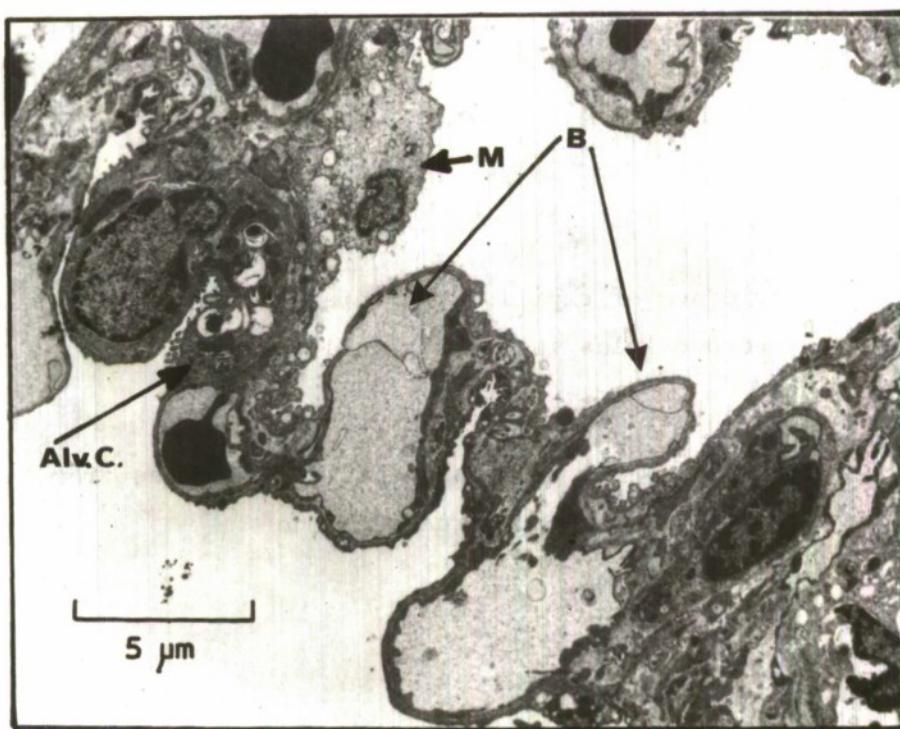


FIG. 13.

Figure 14. A higher magnification of Figure 13. Note the dark line in the basement membrane and the circle of endothelium within the capillary. There appears to be an increase of pinocytotic vesicles in the attenuated cytoplasm of the endothelial cell and there are membrane bound forms between the epithelial and endothelial layers.

Magnification X 24,000

B Ballooning of capillary endothelium  
PV Pinocytotic vesicles  
MBF Membrane bound forms (cytoplasmic fragments)

Figure 15. Evidence of capillary rupture, showing the expulsion of microvesicles into the alveoli.

Magnification X 10,000

E Expulsion of microvesicles from the ruptured membrane

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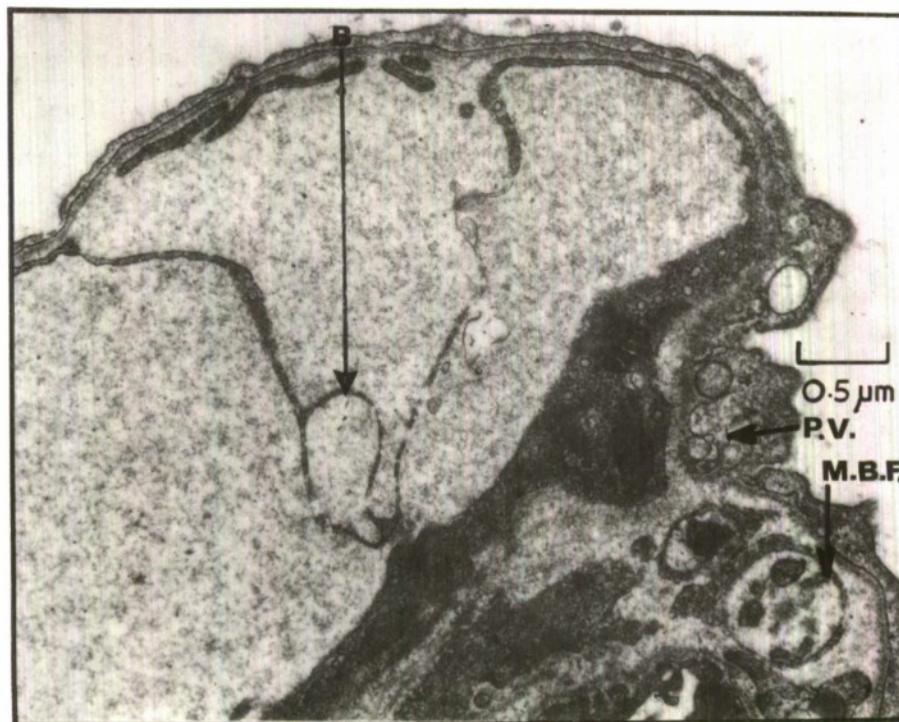


FIG. 14.

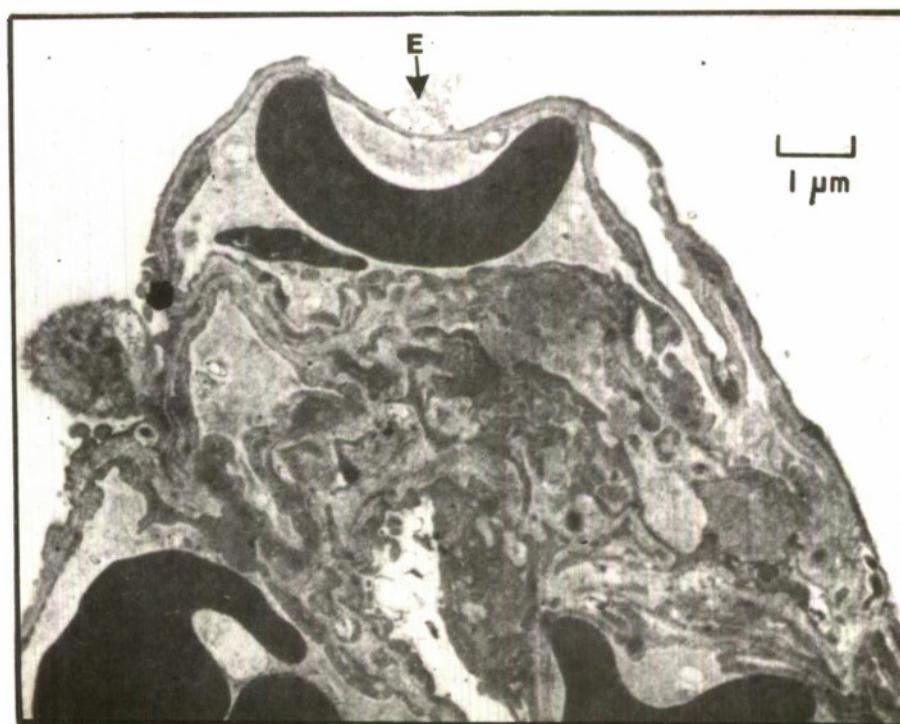


FIG. 15.

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Figure 16. Separation of the pulmonary endothelium with a thrombocyte escaping from the broken capillary wall. Capillary pressure appears to have been responsible for the rupture, also note the separation of the endothelium from the area of the nucleus.

Magnification X 6,200.

T Escaping thrombocyte  
S Separation of the alveolar membrane

Figure 17. Dissolution of the capillary epithelium with fluid and debris contained by its underlying membrane. A mitochondrion appears to be free floating in the capillary.

ALVS Alveolar space  
F. Fluid between the capillary epithelium and basement membrane  
C.E. Capillary epithelium  
C. Capillary  
M. Mitochondrion

Magnification X 15,000

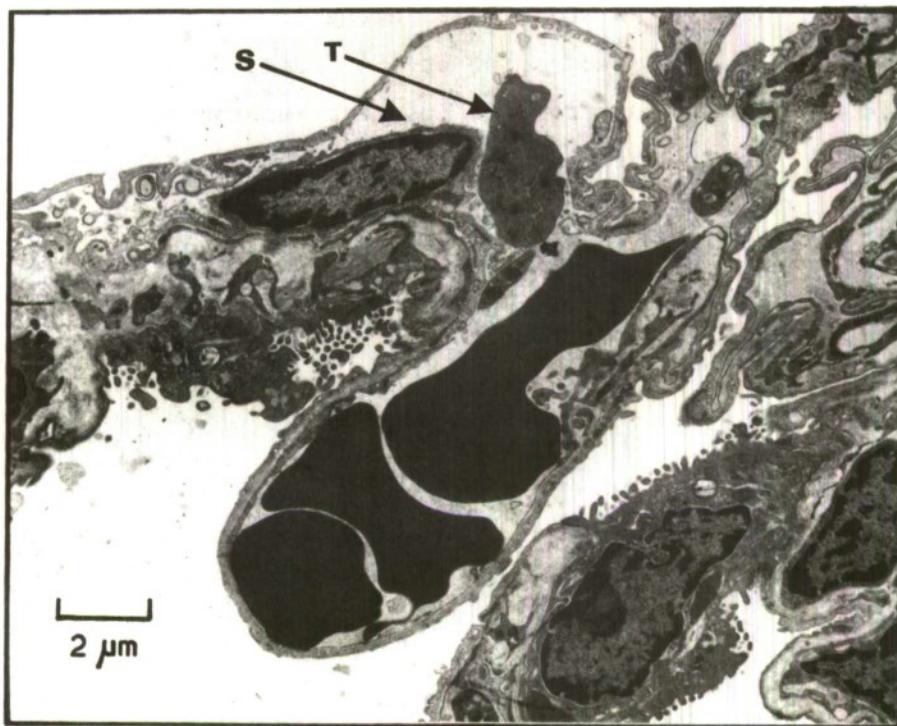


FIG. 16.

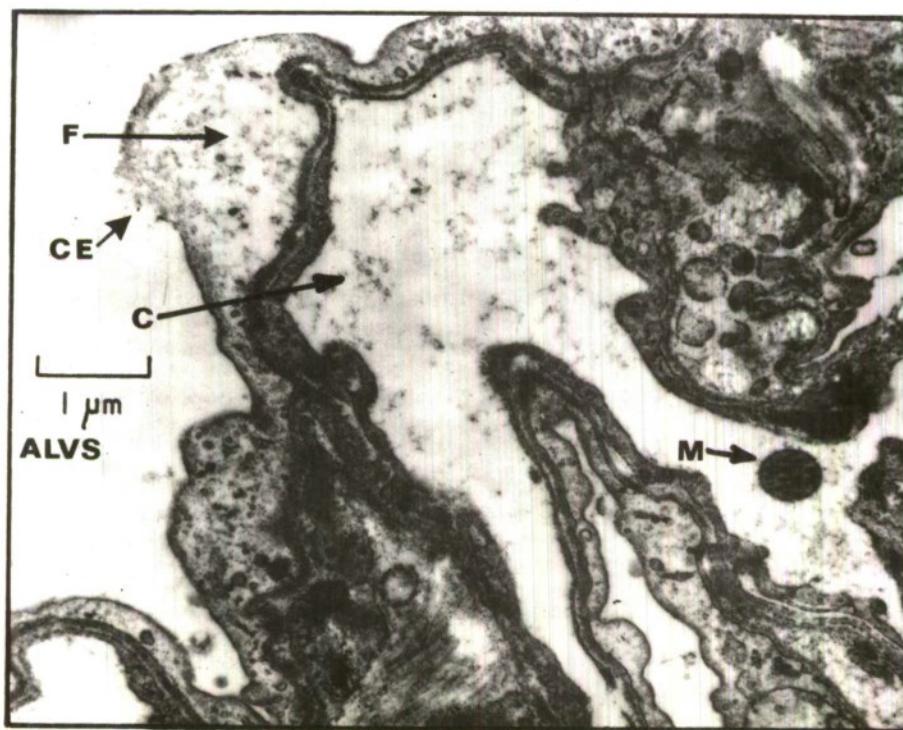


FIG. 17.

Figure 18. Separation and swollen areas of the alveolar septum. The left hand arrow at 'S' indicates separation of the epithelium from its basement membrane. The other arrows point to vacuolation of the endothelium and separation of overlapping endothelial cells. A Type II cell can be seen in the alveolar cleft.

Magnification X 4,000

S	Separation and swollen areas
C	Collagen
ALV.C	Alveolar cell Type II
Wbc	White blood cell
END.C	Endothelial cell

Figure 19. Higher magnification of Figure 18. The top left hand of the picture shows separation of the epithelium. Separation of the endothelium is shown below the red blood cell in the capillary. Note the loop of endothelium in the capillary.

S	Capillary epithelium and endothelium
L	Looping of the endothelium in a capillary
C.	Collagen containing areas of the septum
E.	Elastic tissue

Magnification X 11,000

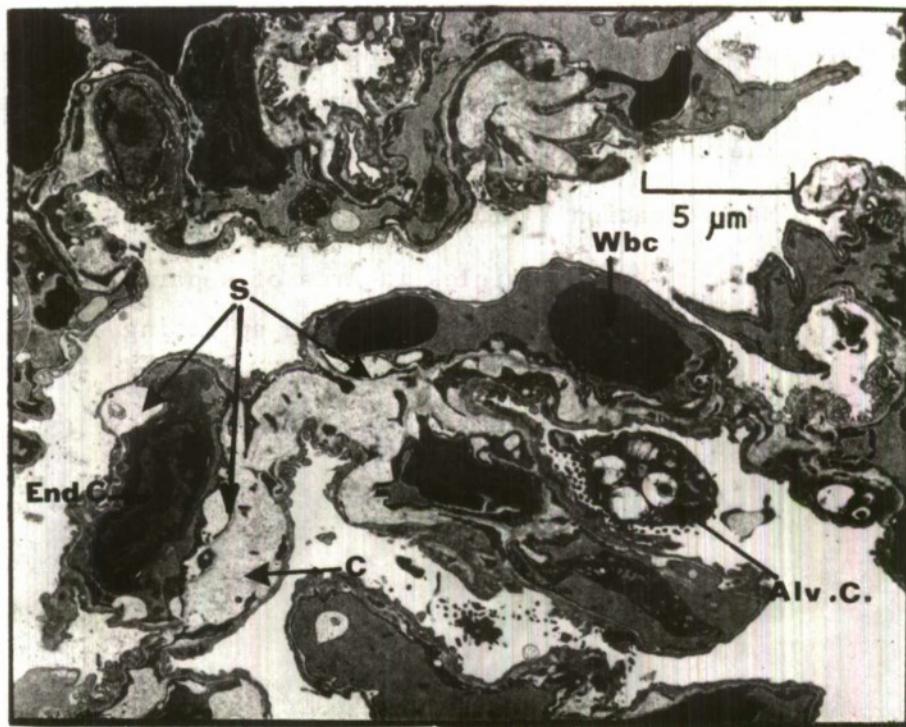


FIG. 18.

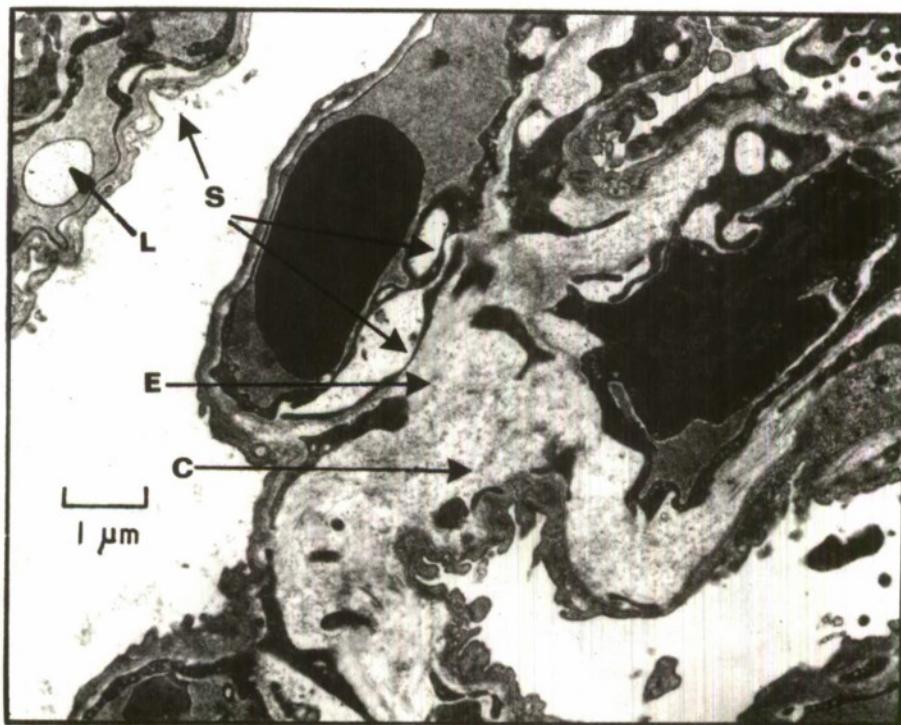


FIG. 19.

Figure 20. Interstitial oedema showing splitting open of the endothelium and basement membrane, note that some of the vesicles from the cytoplasm remain intact.

Magnification X 11,000

P.V. Vesicles in area of separation

B.M. Basement membrane splitting

Figure 21. Higher magnification of Figure 20.

Magnification X 32,000

P.V. Vesicles in area of separation

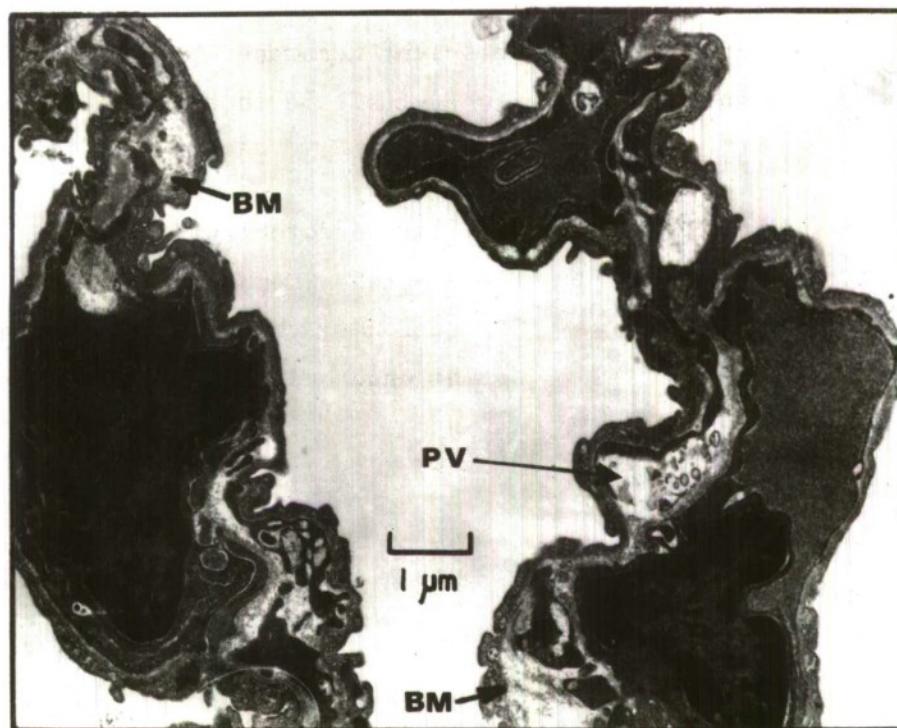


FIG. 20.

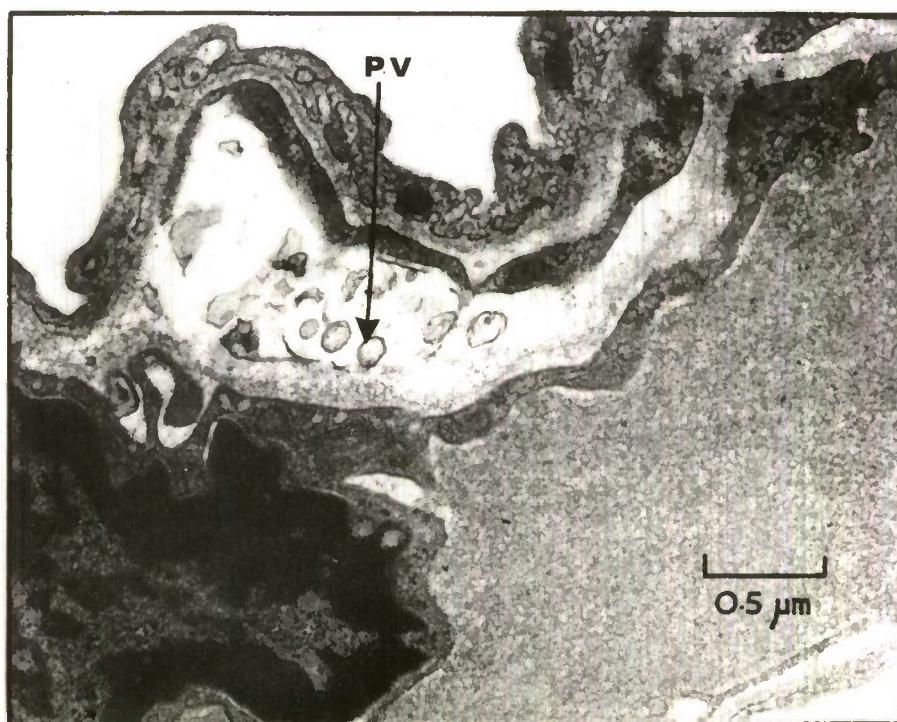


FIG. 21.

Figure 22. Separation of epithelium and endothelium and disintegration of basement membrane. Note the staining of the proteinaceous fluid in the capillary.

Magnification X 18,000.

C.End	Capillary endothelium
F.	Fluid between membrane layers
S.	Separation of the capillary epithelium
END.J.	Normal endothelial junction

Figure 23. Higher magnification of Figure 22. Magnification X 39,000. Notice the debris within the separated region of the epithelium, the disintegration of the basement membrane, and that the junction of the endothelial cell cytoplasm is intact.

S	Separation
F	Fluid
END.J	Endothelial junction

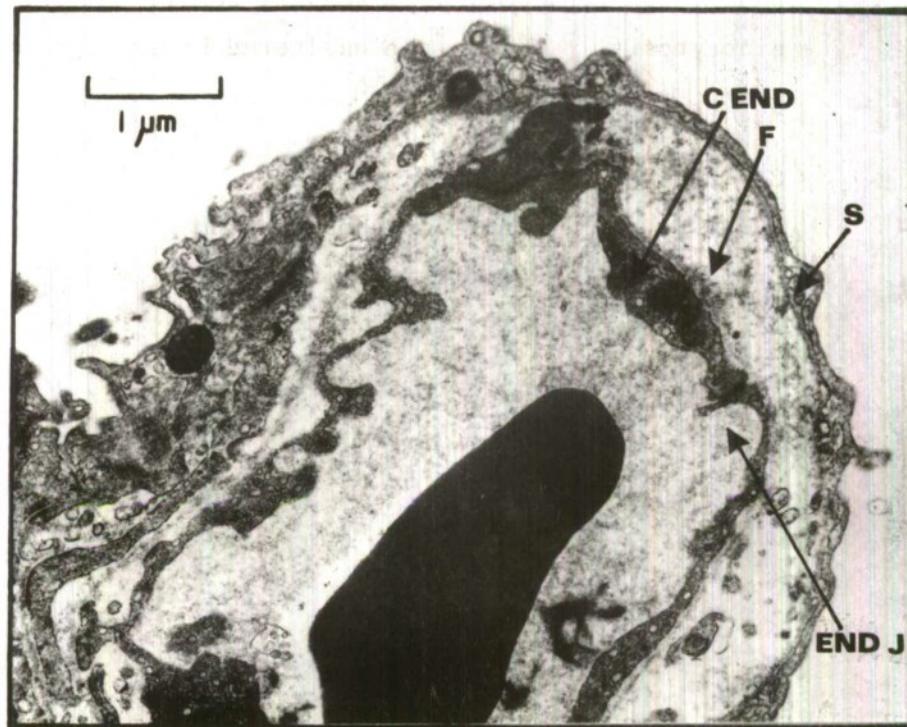


FIG. 22.

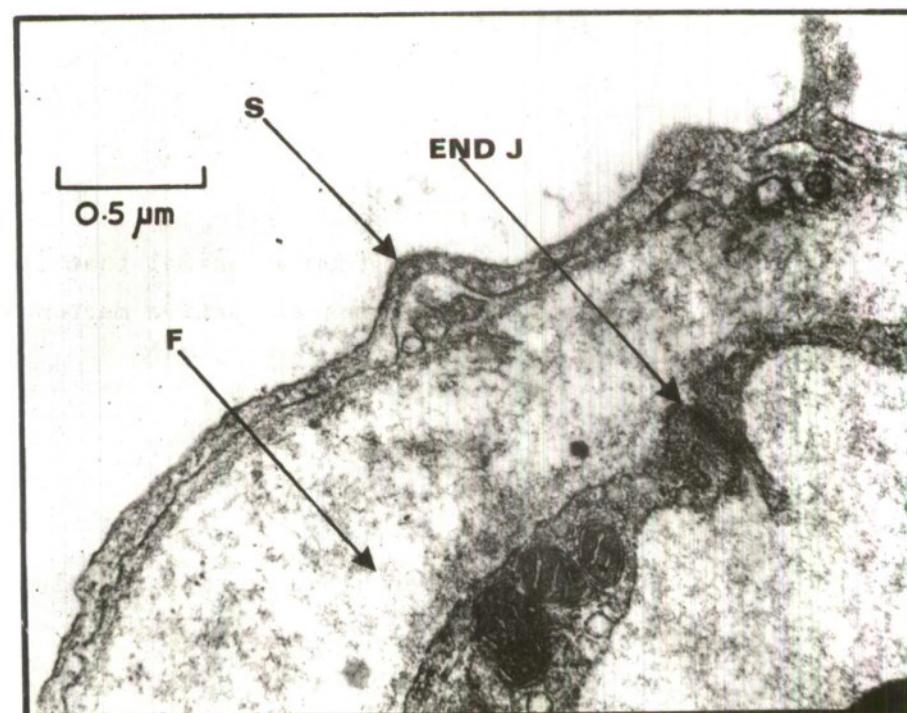


FIG. 23.

Figure 24. Oedematous capillary wall showing debris and increased pinocytosis. Notice the unaltered basement membrane.

Magnification X 34,000

CD	Cytoplasmic debris
C.END	Capillary endothelium
C.EP	Capillary epithelium
C	Capillary

Figure 25. Low magnification of lung from an animal that died showing congested capillaries and active macrophages within the alveoli.

Magnification X 1,300

C.C.	Congested capillary
M	Macrophage
E.	Free red blood cell in the alveolar space

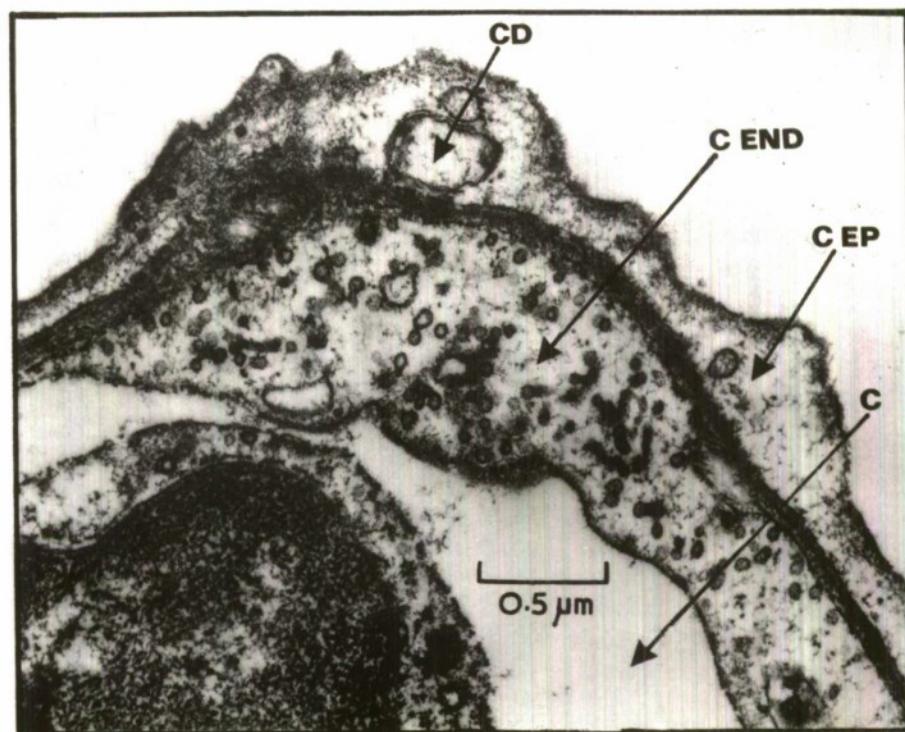


FIG. 24.

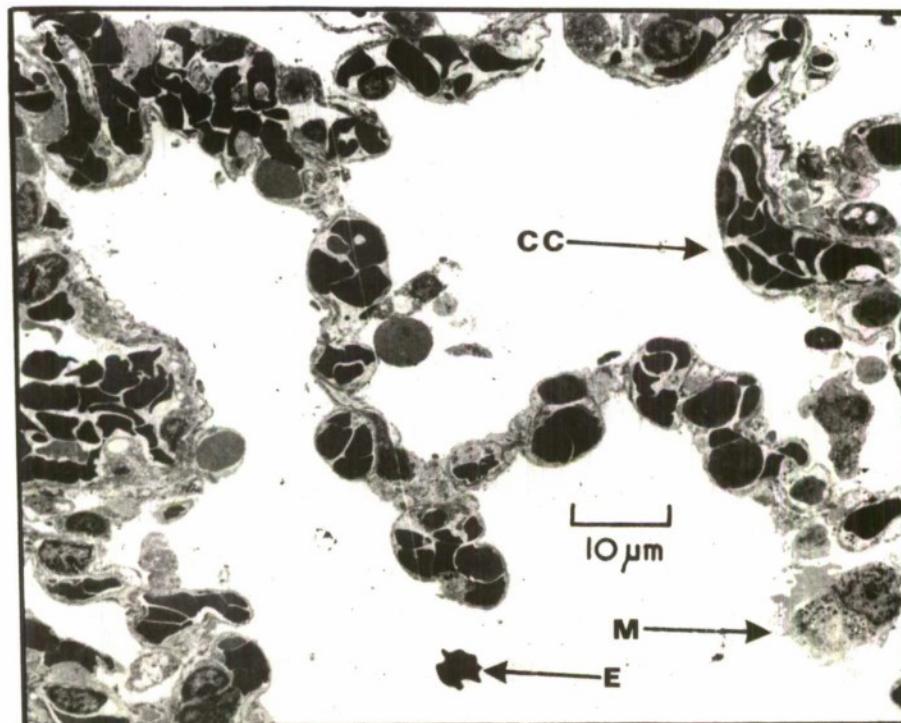


FIG. 25.

Figure 26. Enlargement of Figure 25, showing breakdown of pulmonary epithelium, white blood cells within the lumen of the alveolus, and fluid and debris within the interstitium.

Magnification X 4,700

C.C. Congested capillary  
P.E. Pulmonary epithelium

Figure 27. Slight residual damage in an animal 2 days after exposure.

Magnification X 5,500.

N.M. Normal capillary membrane

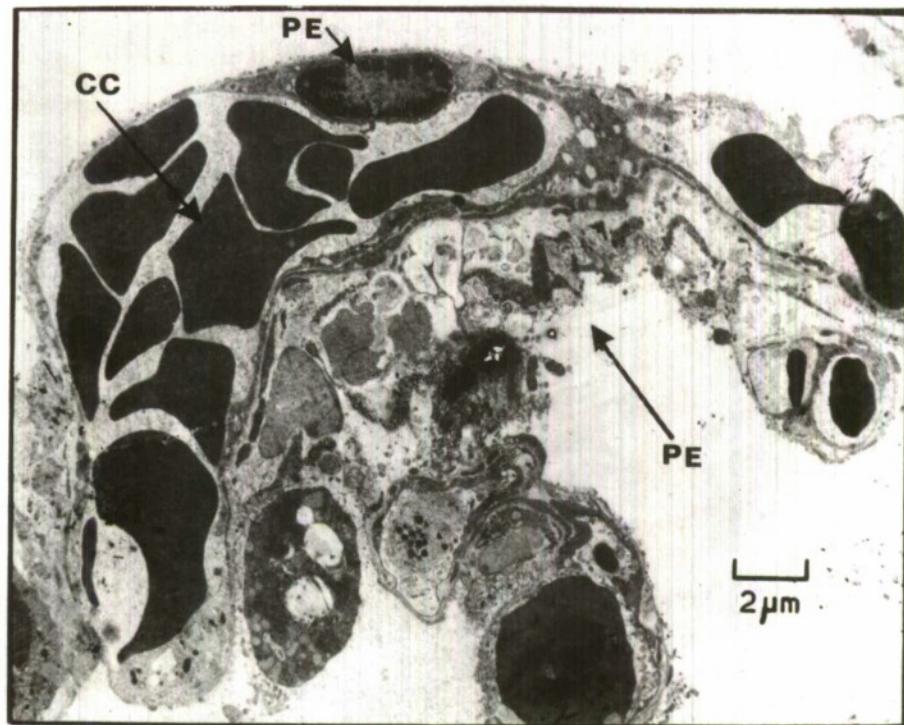


FIG. 26.

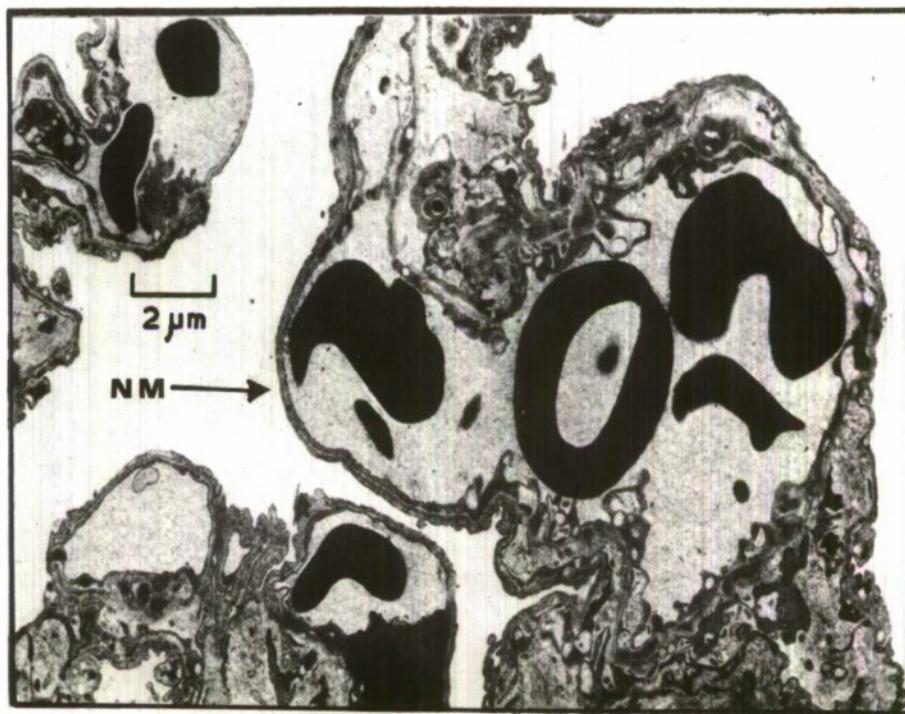


FIG. 27.



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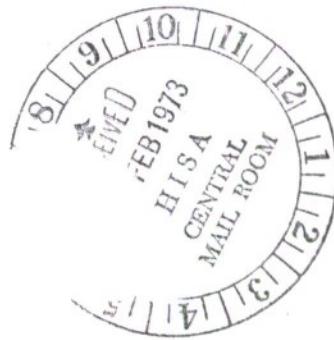
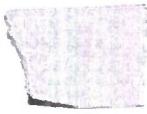
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